Final Evaluation Report

Your Details	
Full Name	Andrés Alejandro Moreira Mendieta
Project Title	Assessing the presence of microplastics in Galapagos sea lions' scats as a bioindicator of pollution in the Galapagos Marine Reserve
Application ID	34036-1
Date of this Report	November 07th, 2022

1. Indicate the level of achievement of the project's original objectives and include any relevant comments on factors affecting this.

Objective	Not achieved	Partially achieved	Fully achieved	Comments
Generate quantitative and qualitative information on marine debris contamination, specifically microplastics, in five rookeries of the Galapagos sea lion (*Zalophus wollebaeki*) in the southeastern Galapagos Marine Reserve.			■	Through an extensive sampling and analysis effort, we identified the presence of microplastics in all the studied populations. We determined quantitative (total particles, average per scat sample, etc.), and qualitative (colour, shape, type of polymer) information. See details of results in Annex 1.
Compare the results obtained between the different populations sampled to evaluate if there are significant differences in relation to the average number of particles found per sample.		■		With the obtained results, general comparisons have been made to measure the level of contamination to which each population is exposed. See details of results in Annex 1. However, I am carrying out, with my team, the formal analysis to evaluate the levels of statistical significance.
Involve local members in the field and laboratory phases of the project.			■	I involved local volunteers in the field and laboratory phase activities, where college students participated in the collecting, managing, and processing of the samples. Rangers of Galapagos National Park also collaborated in the field phase.
Recruit local children and adolescents to spread information about the threats of marine pollution to the local endemic fauna of the Galapagos.			■	I successfully conducted workshops for children and adolescents from a public school of San Cristobal Island through talks and different activities aimed at communicating the threats caused by marine debris to the Galapagos sea lion. See details of photos in Annex 2.

2. Describe the three most important outcomes of your project.

a) Microplastics were found in the scats of Galapagos sea lions, demonstrating the presence of synthetic particles in their food web.

b) The most frequent microplastic shape was microfibers, following the global and regional trends observed regarding microplastic pollution in pinnipeds.

c) Preliminary scientific information was generated that will be used for further research on marine pollution in this endangered species. This effort will lead to a series of actions aimed at mitigating this serious conservation threat in the Galapagos archipelago.

d) The local youth population has shown great interest in participating in actions related to mitigating anthropogenic impacts on native and endemic species, specifically marine debris on the Galapagos sea lion.

3. Explain any unforeseen difficulties that arose during the project and how these were tackled.

This project's only difficulty arose in the laboratory analysis of the samples. Initially, based on the literature, I developed a standard methodology for processing the samples. However, I observed that it was not the most efficient to obtain the best results. Therefore, I tested different chemical reagents until I found the most efficient ones for the chemical digestion of organic material. It was also required to add several steps to the protocol and to build homemade filtration equipment based on literary information. In this way, the difficulties were successfully tackled, but with slightly extended research times and budgeted expenditures.

4. Describe the involvement of local communities and how they have benefitted from the project.

Workshops have been carried out to share the results with children and adolescents from San Cristobal, Galapagos, through theoretical and practical activities. These were carried out in conjunction with researchers and park rangers as conversations to discuss the problems that are affecting the species, in this case, marine debris pollution. Partial results have been shared with the group, ensuring that they understand the consequences of the fragmentation of large debris into microplastics for wildlife and the archipelago overall. Accompanying this activity, we also implemented coastal cleanups and visual census of sea lions. See details of photos in Annex 2.

Also, local volunteers from the Galapagos Academic Institute for the Arts and Sciences (GAIAS) of the Universidad San Francisco de Quito (USFQ) were recruited to collaborate in the field and laboratory phases carried out in San Cristobal, Galapagos. Two students actively participated in these phases, assisting with the field collection and laboratory processing of scat samples, and other activities related to the project. These activities involved volunteers understanding and learning about the problem of marine pollution in an endemic and emblematic species, and its

implications for conservation, not only theoretically, but also in a practical and participatory approach. Similarly, several park rangers from the Galapagos National Park participated in the expeditions, not only as control authorities but also as members of the team, playing an important role in the collection of samples.

5. Are there any plans to continue this work?

Yes, based on the results obtained and the growing global concern about plastic debris pollution, we are planning to extend the research to the west of the archipelago (Isabela and Fernandina islands). Furthermore, for this research, we will rely on a protocol that, in addition to quantifying microplastics, employs novel DNA-metabarcoding technology to identify the prey of our target species. This will allow us to relate the diet of the Galapagos sea lion to the occurrence of microplastics, and thus to elaborate possible routes of indirect incorporation of these particles (e.g., which are the main prey, and which are the most susceptible to transferring microplastics).

We estimate the logistical planning of this project in the first quarter of 2023, with a tentative start of the field phase in early June 2023. To carry out this project, however, funding with a 2nd Rufford Small Grant will be critical. To this end, we have a proposal ready for submission once this report is reviewed and approved.

6. How do you plan to share the results of your work with others?

The results presented here are being enhanced with further statistical analysis with the aim of being published as a high impact scientific publication. This research will set a precedent in academia, which will trigger further studies of microplastic pollution in the Galapagos Islands and its effects on biodiversity. It is expected that authorities of the State of Ecuador, through the Directorate of the Galapagos National Park, will be notified of the results of this research through a colloquium that I will give at the Galapagos Science Center on San Cristobal Island.

In May 2023, this research, which serves as my thesis for the Master's programme in Tropical Ecology and Conservation at USFQ, will be presented to the public as a colloquium in Quito, Ecuador. This presentation will be attended by academics from various research areas, undergraduate and graduate students, and anyone in general who wishes to attend.

Also, as this is an investigation with impacting results for an endemic and threatened species, and carried out in pristine ecosystems, it is expected to attract the attention of several local and national media to communicate the results and generate awareness in the Ecuadorian population. We plan to present these results at future congresses of organizations focused on the study of marine mammals.

7. Looking ahead, what do you feel are the important next steps?

The next step is to complete the writing of the scientific publication so that it can be published in a high impact journal, and afterward, to divulge the results. Then, it is essential to broaden the scope of the research and start executing the next project

in the western populations of the archipelago to have a holistic and comprehensive understanding of this threat to the species.

8. Did you use The Rufford Foundation logo in any materials produced in relation to this project? Did the Foundation receive any publicity during the course of your work?

So far, I have used the Rufford Foundation logo for presentations given to students and teachers in my graduate program at USFQ, as well as to GAIAS academics.

In the future, it is planned to continue recognising Rufford Foundation's vital contribution by placing the logo on all presentations and activities linked to this project, and future projects that we have in common.

It is also worth reporting that, because my professors were aware of my accreditation to this grant for my research, they encouraged students in my programme to apply for these funds to finance their master's research and strengthen their fundraising skills, which are invaluable aspects in the early training of scientists.

9. Provide a full list of all the members of your team and their role in the project.

Dr. Diego Páez-Rosas (USFQ-GSC) fulfilled the role of co-director of my master's thesis in Tropical Ecology and Conservation, providing research permits to work within the protected areas of the Galapagos National Park, and at the GSC facilities in San Cristobal and the USFQ campus in Quito, Ecuador. He also contributed financially and intellectually in all stages of the research (logistics, field and laboratory phases, data analysis, and paper writing and review).

Dr. Odei García-Garín (Universitat de Barcelona) fulfilled the role of external co-director of my master's thesis in Tropical Ecology and Conservation, providing permits to work in the facilities of the Univesitat de Barcelona, Spain, to carry out the polymer characterization by μFT-IR spectrometry. He also contributed intellectually in several stages of the research (field and laboratory phases, data analysis, and paper writing and review).

Dr. Asunción Borrell (Universitat de Barcelona) fulfilled the role of research advisor, providing financial support to cover the costs of polymer analysis by μFT-IR spectrometry at the Universitat de Barcelona. She also contributed intellectually in other phases of the research (data analysis, and paper writing and review).

Dr. Massimiliano Drago (Universitat de Barcelona) fulfilled the role of research advisor, contributing intellectually to several stages of the research (data analysis, and paper writing and review).

Juan Pablo Muñoz-Pérez (USFQ-GSC) fulfilled the role of research advisor, contributing intellectually to several stages of the research (field and laboratory phases, data analysis, and paper review).

Diego Urquía (USFQ-GSC) fulfilled the role of research advisor, contributing physically and intellectually in the logistics and sample collection for the field phase. He also

contributed intellectually to other stages of the research (data analysis, and paper review).

10. Any other comments?

As I mentioned previously, the results presented in this report are preliminary and are being rigorously statistically analysed to be published in a scientific publication in a high impact journal. However, they are not yet final, and the urgency in sharing this information with The Rufford Foundation is that, because of the proximity to 2023, we have contemplated the need to start planning the extension of this research to new regions of the archipelago, where it is necessary to continue producing information.

Therefore, I will briefly present my findings in Annex 1.

ANNEX 1

A total of 180 scat samples of the Galapagos sea lion (*Zalophus wollebaeki*) were collected from five rookeries on four islands in southeastern Galapagos (*Figure 1*) between August and September 2021, as follows:

- El Malecón (San Cristobal): 60 samples.
- Punta Pitt (San Cristobal): 30 samples.
- Bahía de Santa Fe (Santa Fe): 30 samples.
- Punta Suarez (Española): 30 samples.
- Post Office (Floreana): 30 samples.

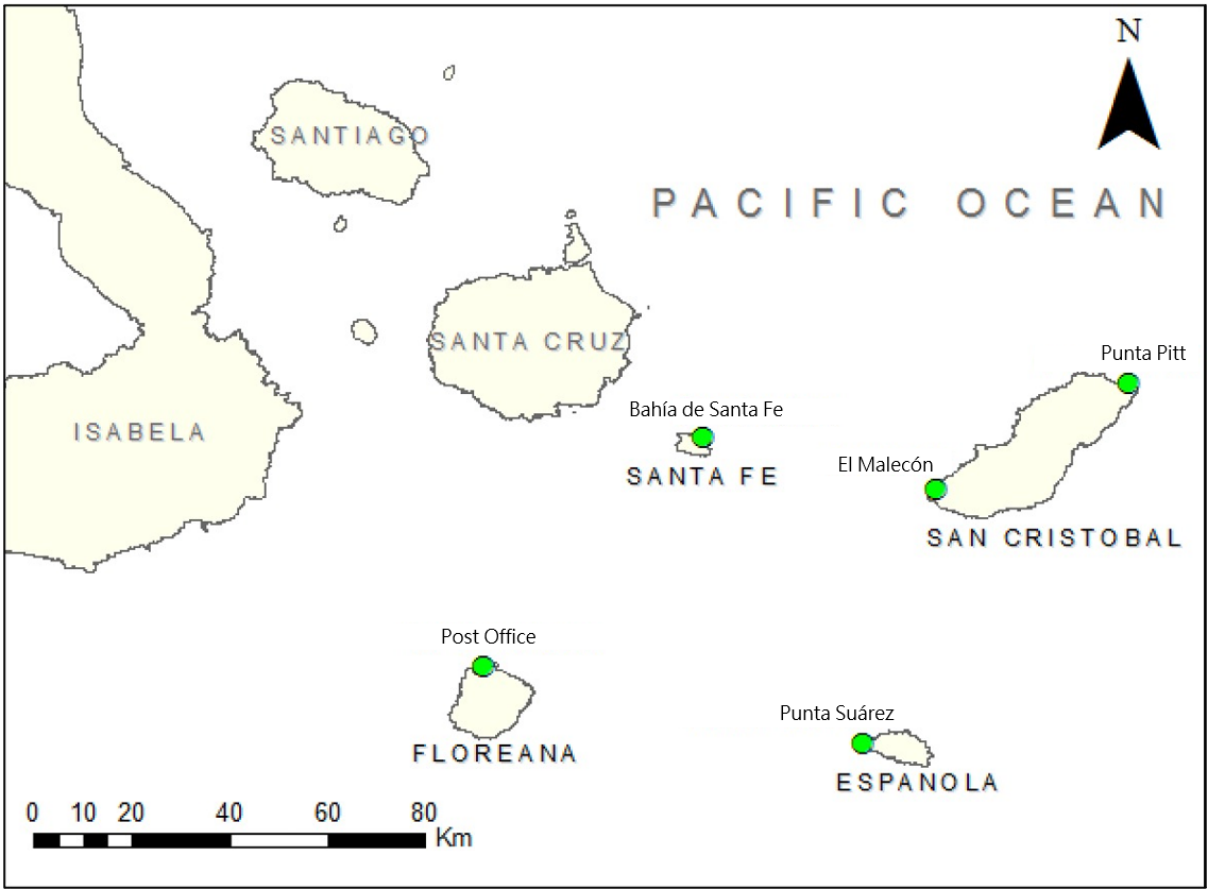


Figure 1. Sampled rookeries in the southeastern Galapagos Islands.

Potential microplastics recovered

The laboratory phase was carried out at USFQ and Universitat de Barcelona between November 2021 and September 2022. A total of 259 potential microplastic particles were found in 121 Galapagos sea lion scats of the 180 collected (67.2%). "El Malecón" rookery, which is the largest in the entire archipelago and located on San Cristobal Island, had the greatest sampling effort (n=60), in which 126 potential microplastics were found. This was followed by the "Punta Pitt" rookery, also on San Cristobal, with 40 potential particles. Then, in the "Punta Suarez" rookery, 34 particles were found; in "Post Office", 33; and in "Bahia de Santa Fe", 26. *Figure 2* shows this information, with

the data segmented by microplastic shape. Fibers were the most frequent microplastic shape found at all sites.

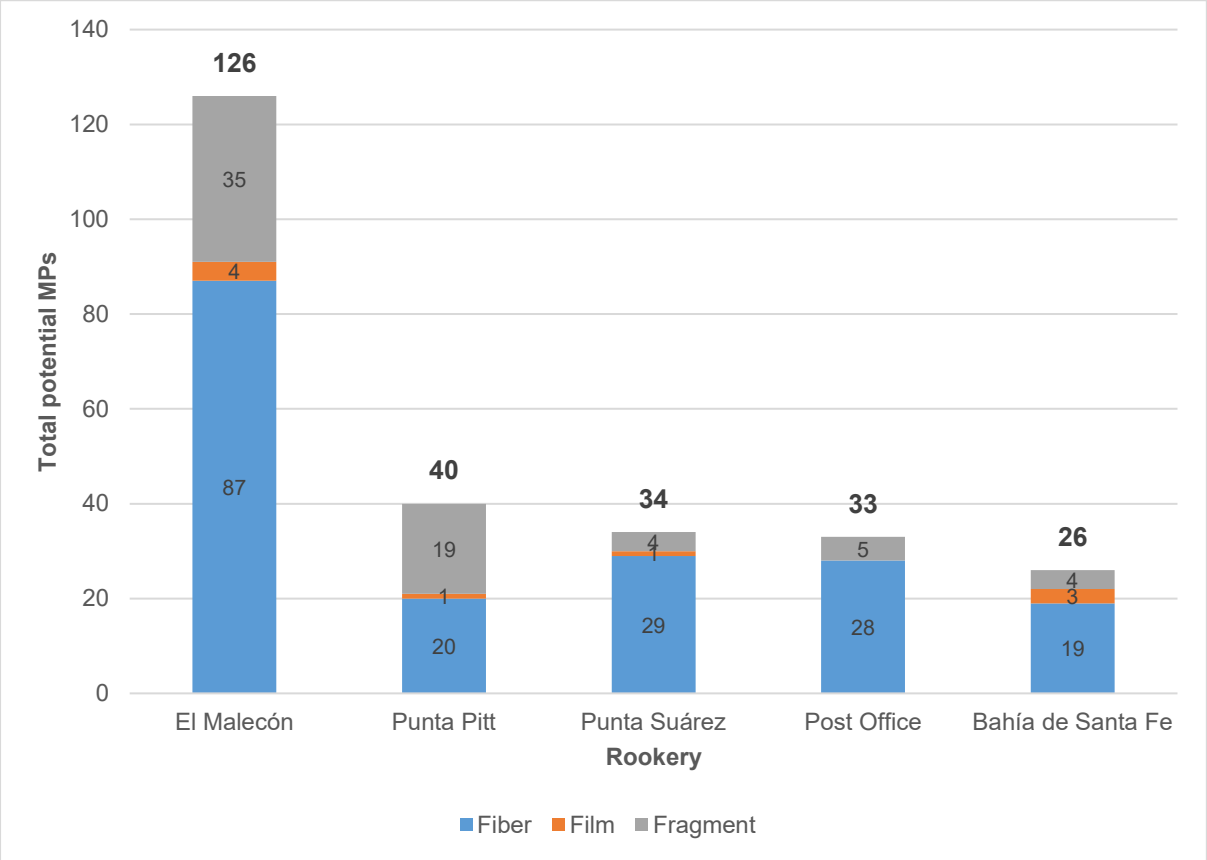


Figure 2. Total potential microplastics (MPs) retrieved at each rookery, segmented by shape.

To better represent the data, and to eliminate the bias of different sampling efforts, the means of potential microplastics recovered per sample in each rookery were calculated. Thus, in "El Malecón", 2.10 potential particles per sample were obtained; in "Punta Pitt", 1.33 particles per sample; in "Punta Suárez", 1.13 particles per sample; in "Post Office", 1.10 particles per sample; and, in "Bahía de Santa Fe", 0.87 particles per sample. *Figure 3* shows this information, with the data segmented according to the mean number of microplastic shapes found per rookery. Again, fibers were the most frequent microplastic shape found, on average, at all sites.

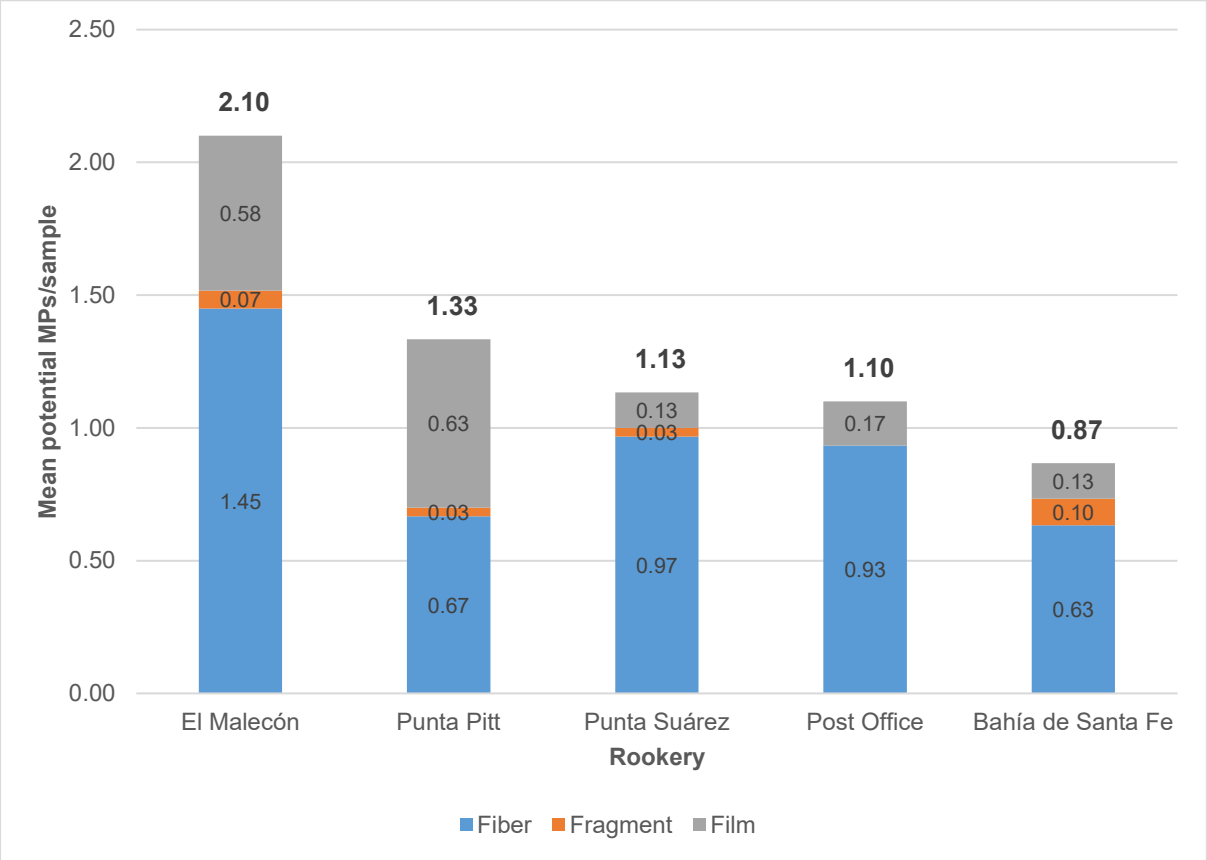


Figure 3. Mean potential MPs per collected sample at each rookery, segmented by shape.

Polymer characterization by μFT-IR Spectrometry

Potential microplastic particles, as the name implies, are not considered as confirmed microplastics until they have undergone a μFT-IR spectrometry process that confirms that their composition is synthetic polymer-based. A total of 53 microplastics composed of polymers were confirmed after μFT-IR spectrometry results and adjustment with the cross-contamination protocol. "El Malecón" rookery was the one with the highest total of microplastics, with 20 particles. Following this, were the rookeries "Punta Suárez", with 12 confirmed particles; "Punta Pitt", with 8 confirmed particles; "Bahía de Santa Fe", with 7 confirmed particles; and "Post Office", with 6 confirmed particles. *Figure 4* shows this information, with the data segmented by microplastic shape. Fibers were the most frequent microplastic shape found at all sites.

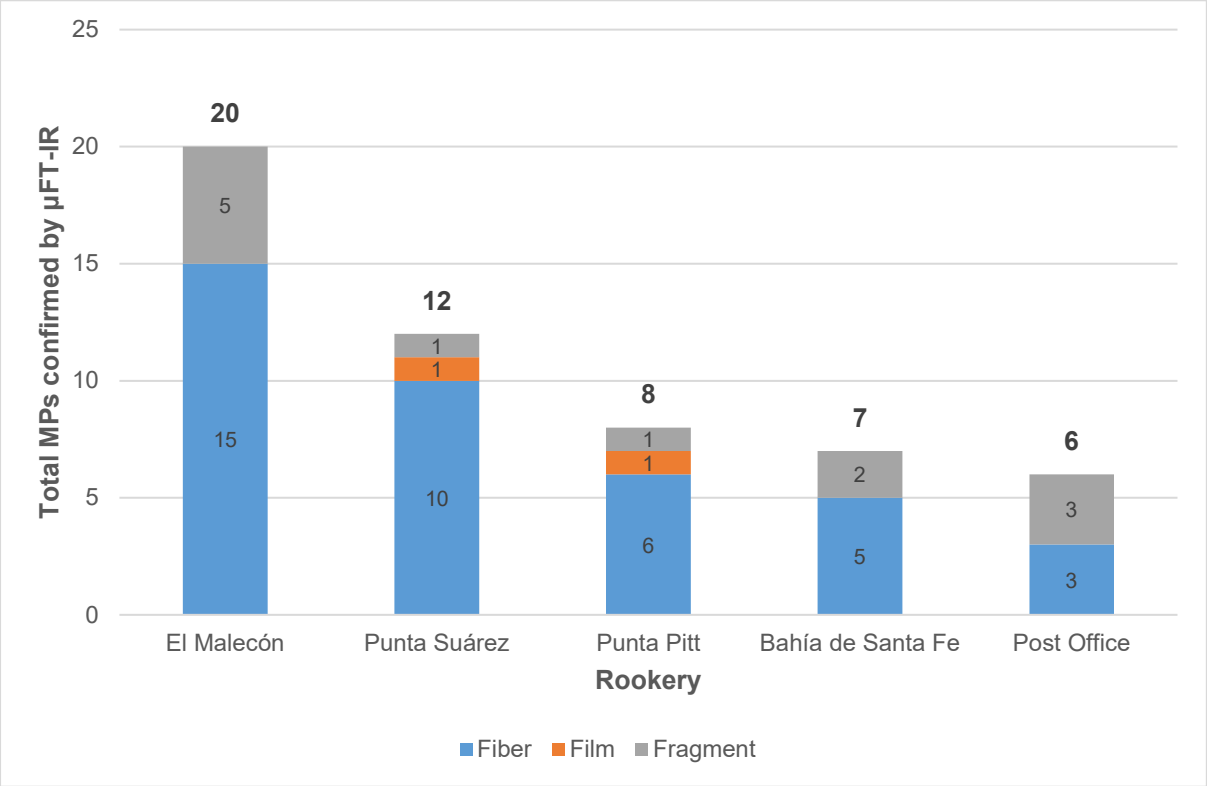


Figure 4. Total µFT-IR confirmed MPs at each rookery, segmented by shape.

Similarly, to eliminate the sampling effort bias, the mean number of confirmed microplastics per sample was calculated for each rookery. Thus, "Punta Súarez" rookery was the one with the highest average of microplastics per sample, 0.40 particles per scat sample. This was followed by the rookeries "El Malecón", with 0.33 particles per sample; "Punta Pitt", with 0.27 particles per sample; "Bahía de Santa Fe", with 0.23 particles per sample; and "Post Office" with 0.20 particles per sample. *Figure 5* shows this information, with the data segmented according to the mean number of microplastic shapes found per rookery. Fibers were the most frequent form of microplastic found, on average, at all sites.

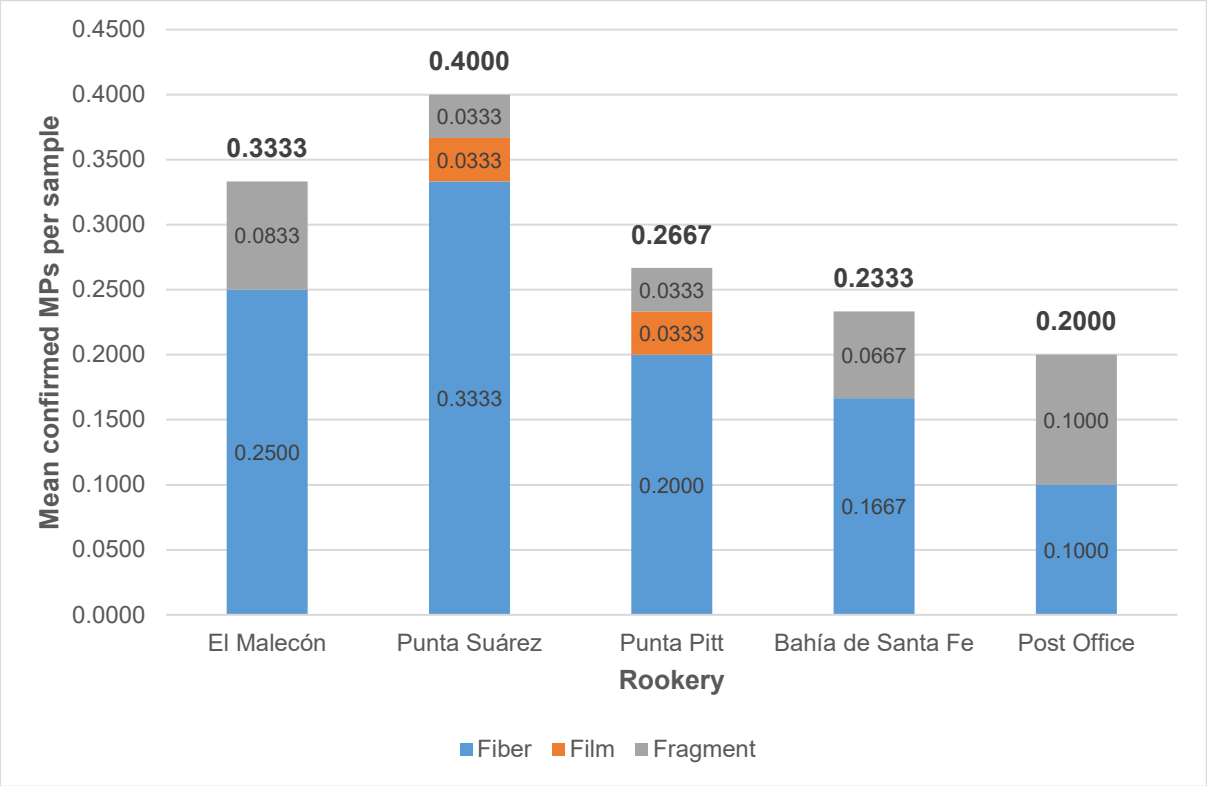


Figure 5. Mean µFT-IR confirmed MPs per sample at each rookery, segmented by shape

Finally, as for polymer composition by site, 12 different polymer types were identified in total, with polypropylene-polyethylene copolymer being the most common overall. "El Malecón" rookery had the highest polymer diversity, with eight types, polypropylene being the dominant polymer. "Punta Suárez" was composed of six different polymer types, with cellulose being the dominant. "Punta Pitt" was composed of seven different polymers, with cellulose being the dominant. "Bahía de Santa Fe" was composed of three polymers, with cellophane and polypropylene-polyethylene copolymer being the most representative. Finally, "Post Office" was composed of four polymers, with polypropylene-polyethylene copolymer and cellulose being the most representatives. This information is shown in *Figure 6* and *Figure 7*.

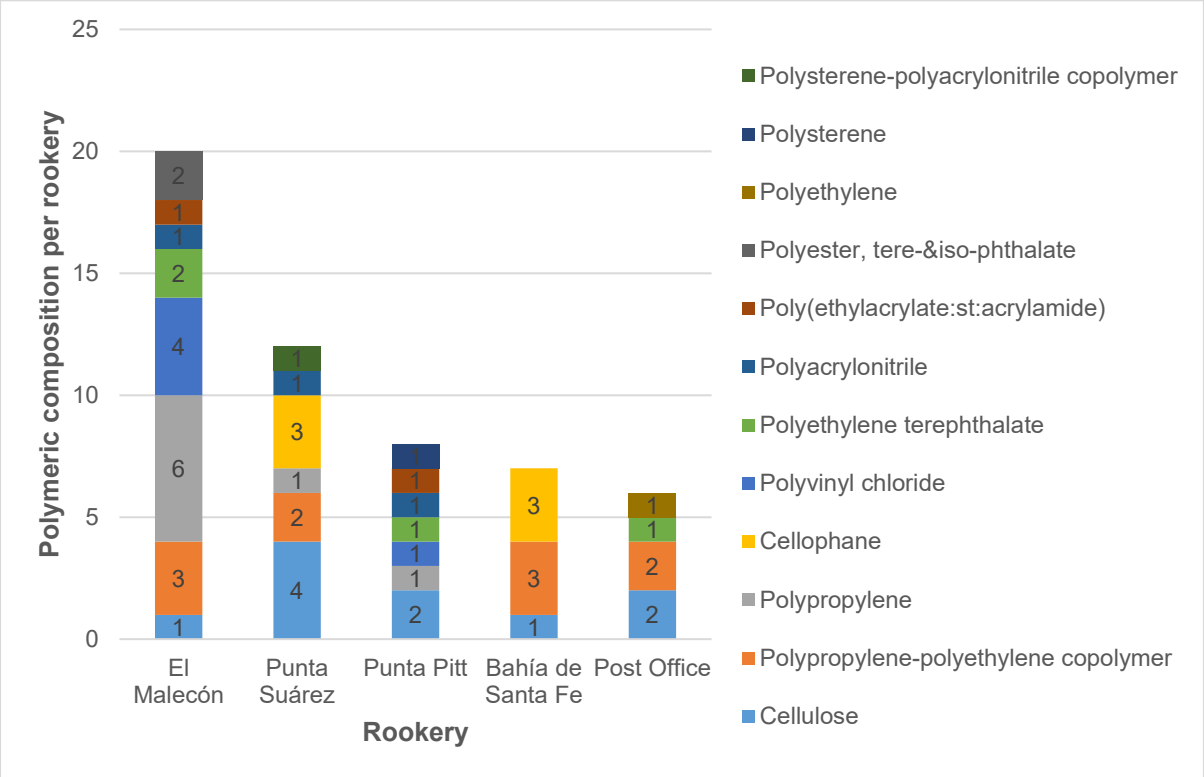


Figure 6. Total μFT-IR confirmed MPs at each rookery, segmented by polymer type

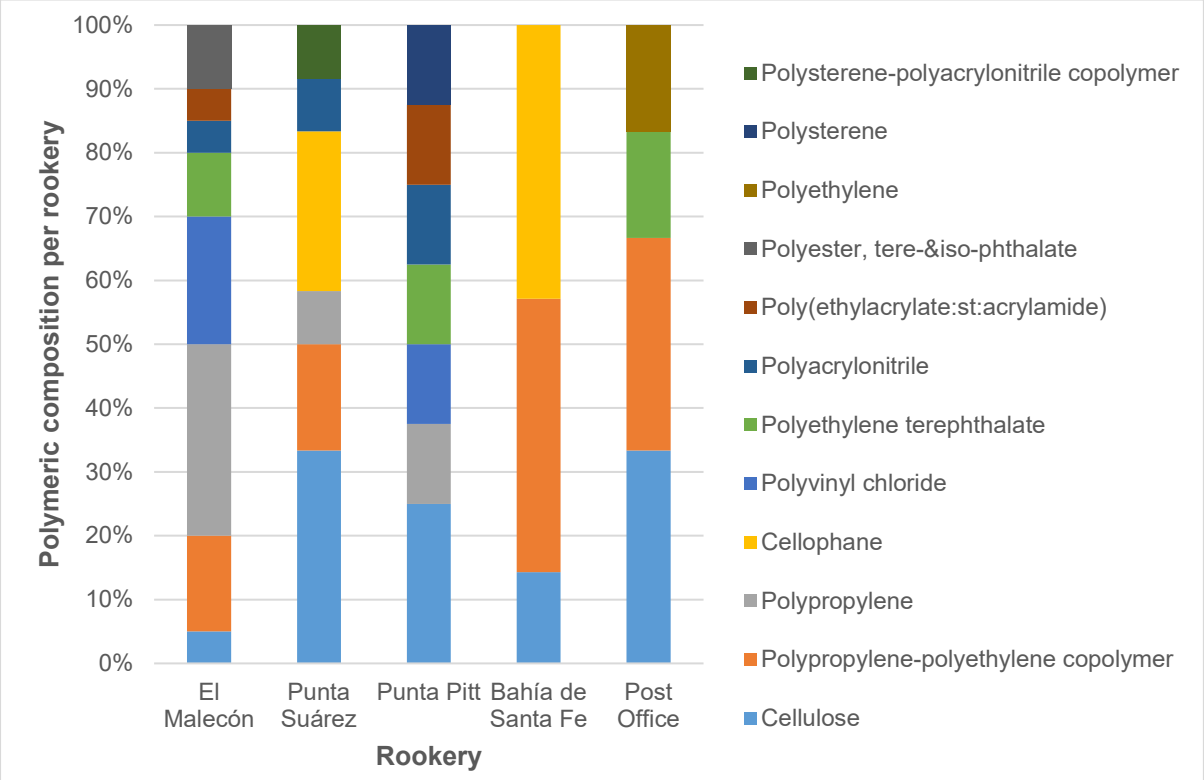


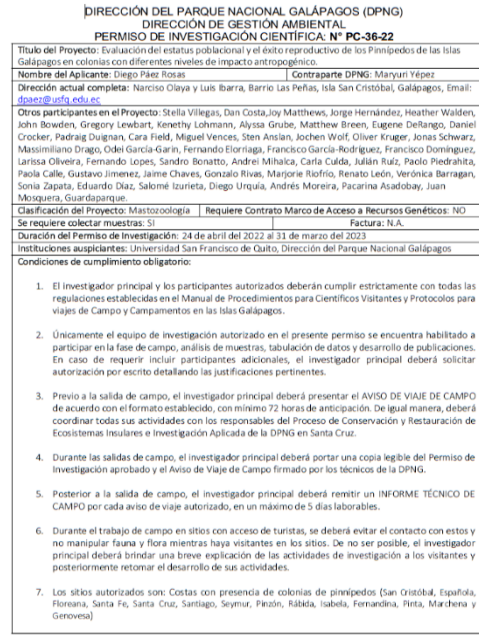
Figure 7. Observed polymeric composition at each rookery.

ANNEX 2

Images below show a campaign that was conducted with nine teenagers in Punta Pitt, San Cristobal. In this campaign, I was able to explain to the students about marine debris pollution and its implications for the endemic fauna of the archipelago, as well as the preliminary results of my research at the Punta Pitt rookery. Then, we carried out a coastal cleanup and a census of the individuals in this important Galapagos sea lion rookery, which coincides with one of my sampling sites.

ANNEX 3

Current research permits certifying that the samples collected for this research were merely for scientific purposes. This permit is the result of an inter-institutional collaboration between the Galapagos National Park Directorate, Universidad San Francisco de Quito, Galapagos Science Center, and The Rufford Foundation.



DIRECCIÓN DEL PARQUE NACIONAL GALÁPAGOS (DPNG)
DIRECCIÓN DE GESTIÓN AMBIENTAL
PERMISO DE INVESTIGACIÓN CIENTÍFICA: **N° PC-36-22**

Título del Proyecto: Evaluación del estatus poblacional y el éxito reproductivo de los Pinnípedos de las Islas Galápagos en colonias con diferentes niveles de impacto antropogénico.

Nombre del Aplicante: Diego Páez Rosas | **Contraparte DPNG:** Maryuri Yépez

Dirección actual completa: Narciso Olaya y Luis Ibarra, Barrio Las Peñas, Isla San Cristóbal, Galápagos, Email: dpaez@usfq.edu.ec

Otros participantes en el Proyecto: Stella Villegas, Dan Costa,Joy Matthews, Jorge Hernández, Heather Walden, John Bowden, Gregory Lewbart, Kenethy Lohmann, Alyssa Grube, Matthew Breen, Eugene DeRango, Daniel Crocker, Padraig Duignan, Cara Field, Miguel Vences, Sten Anslan, Jochen Wolf, Oliver Kruger, Jonas Schwarz, Massimiliano Drago, Odei García-Garín, Fernando Elorriaga, Francisco García-Rodríguez, Francisco Domínguez, Larissa Oliveira, Fernando Lopes, Sandro Bonatto, Andrei Mihalca, Carla Culda, Julián Ruiz, Paolo Piedrahita, Paola Calle, Gustavo Jimenez, Jaime Chaves, Gonzalo Rivas, Marjorie Riofrío, Renato León, Verónica Barragan, Sonia Zapata, Eduardo Díaz, Salomé Izurieta, Diego Urquía, Andrés Moreira, Pacarina Asadobay, Juan Mosquera, Guardaparque.

Clasificación del Proyecto: Mastozoología | **Requiere Contrato Marco de Acceso a Recursos Genéticos:** NO

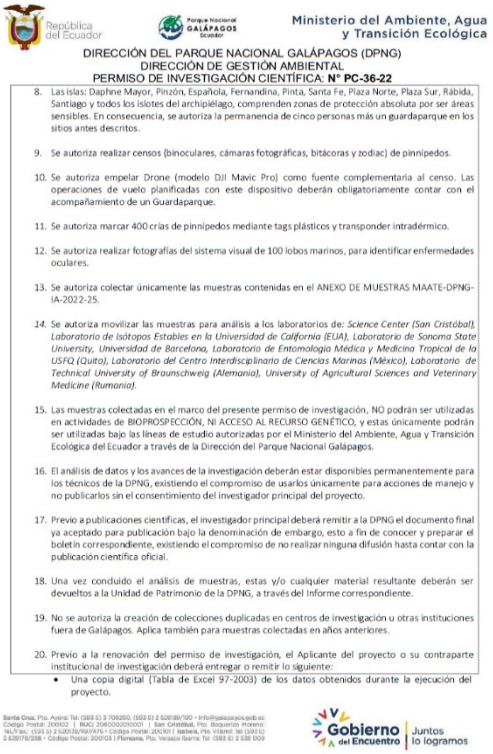
Se requiere colectar muestras: SI | **Factura:** N.A.

Duración del Permiso de Investigación: 24 de abril del 2022 al 31 de marzo del 2023

Instituciones auspiciantes: Universidad San Francisco de Quito, Dirección del Parque Nacional Galápagos

Condiciones de cumplimiento obligatorio:

1. El investigador principal y los participantes autorizados deberán cumplir estrictamente con todas las regulaciones establecidas en el Manual de Procedimientos para Científicos Visitantes y Protocolos para viajes de Campo y Campamentos en las Islas Galápagos.
2. Únicamente el equipo de investigación autorizado en el presente permiso se encuentra habilitado a participar en la fase de campo, análisis de muestras, tabulación de datos y desarrollo de publicaciones. En caso de requerir incluir partidpantes adicionales, el investigador principal deberá solicitar autorización por escrito detallando las justificaciones pertinentes.
3. Previo a la salida de campo, el investigador principal deberá presentar el AVISO DE VIAJE DE CAMPO de acuerdo con el formato establecido, con mínimo 72 horas de anticipación. De igual manera, deberá coordinar todas sus actividades con los responsables del Proceso de Conservación y Restauración de Ecosistemas Insulares e Investigación Aplicada de la DPNG en Santa Cruz.
4. Durante las salidas de campo, el investigador principal deberá portar una copia legible del Permiso de Investigación aprobado y el Aviso de Viaje de Campo firmado por los técnicos de la DPNG.
5. Posterior a la salida de campo, el investigador principal deberá remitir un INFORME TÉCNICO DE CAMPO por cada aviso de viaje autorizado, en un máximo de 5 días laborables.
6. Durante el trabajo de campo en sitios con acceso de turistas, se deberá evitar el contacto con estos y no manipular fauna y flora mientras haya visitantes en los sitios. De no ser posible, el investigador principal deberá brindar una breve explicación de las actividades de investigación a los visitantes y posteriormente retomar el desarrollo de sus actividades.
7. Los sitios autorizados son: Costas con presencia de colonias de pinnípedos (San Cristóbal, Española, Floreana, Santa Fe, Santa Cruz, Santiago, Seymur, Pinzón, Rábida, Isabela, Fernandina, Pinta, Marchena y Genovesa)



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8. Las islas: Daphne Mayor, Pinzón, Española, Fernandina, Pinta, Santa Fe, Plaza Norte, Plaza Sur, Rábida, Santiago y todos los islotes del archipiélago, comprenden zonas de protección absoluta por ser áreas sensibles. En consecuencia, se autoriza la permanencia de cinco personas más un guardaparque en los sitios antes descritos.
9. Se autoriza realizar censos (binoculares, cámaras fotográficas, bitácoras y zodiac) de pinnípedos.
10. Se autoriza empelar Drone (modelo DJI Mavic Pro) como fuente complementaria al censo. Las operaciones de vuelo planificadas con este dispositivo deberán obligatoriamente contar con el acompañamiento de un Guardaparque.
11. Se autoriza marcar 400 crías de pinnípedos mediante tags plásticos y transponder intradérmico.
12. Se autoriza realizar fotografías del sistema visual de 100 lobos marinos, para identificar enfermedades oculares.
13. Se autoriza colectar únicamente las muestras contenidas en el ANEXO DE MUESTRAS MAATE-DPNG-IA-2022-25.
14. Se autoriza movilizar las muestras para análisis a los laboratorios de: *Science Center (San Cristóbal), Laboratorio de Isótopos Estables en la Universidad de California (EUA), Laboratorio de Sonoma State University, Universidad de Barcelona, Laboratorio de Entomología Médica y Medicina Tropical de la USFQ (Quito), Laboratorio del Centro Interdisciplinario de Ciencias Marinas (México), Laboratorio de Technical University of Braunschweig (Alemania), University of Agricultural Sciences and Veterinary Medicine (Rumania).*
15. Las muestras colectadas en el marco del presente permiso de investigación, NO podrán ser utilizadas en actividades de BIOPROSPECCIÓN, NI ACCESO AL RECURSO GENÉTICO, y estas únicamente podrán ser utilizadas bajo las líneas de estudio autorizadas por el Ministerio del Ambiente, Agua y Transición Ecológica del Ecuador a través de la Dirección del Parque Nacional Galápagos.
16. El análisis de datos y los avances de la investigación deberán estar disponibles permanentemente para los técnicos de la DPNG, existiendo el compromiso de usarlos únicamente para acciones de manejo y no publicarlos sin el consentimiento del investigador principal del proyecto.
17. Previo a publicaciones científicas, el investigador principal deberá remitir a la DPNG el documento final ya aceptado para publicación bajo la denominación de embargo, esto a fin de conocer y preparar el boletín correspondiente, existiendo el compromiso de no realizar ninguna difusión hasta contar con la publicación científica oficial.
18. Una vez concluido el análisis de muestras, estas y/o cualquier material resultante deberán ser devueltos a la Unidad de Patrimonio de la DPNG, a través del Informe correspondiente.
19. No se autoriza la creación de colecciones duplicadas en centros de investigación u otras instituciones fuera de Galápagos. Aplica también para muestras colectadas en años anteriores.
20. Previo a la renovación del permiso de investigación, el Aplicante del proyecto o su contraparte institucional de investigación deberá entregar o remitir lo siguiente:
    - Una copia digital (Tabla de Excel 97-2003) de los datos obtenidos durante la ejecución del proyecto.

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- Dos conferencias para los guardaparques de la DPNG, guías naturalistas y otras personas interesadas sobre los avances del proyecto. Para establecer el cronograma se deberá coordinar con el Responsable de Investigación Aplicada de la DPNG.
- Un INFORME DE AVANCES del proyecto, con todos los detalles de la investigación y sus recomendaciones técnicas aplicables al manejo de las Áreas Protegidas.
- Artículos y publicaciones resultantes del proyecto de investigación: en el caso de publicaciones únicamente en formato digital; si son libros o tesis, 1 impresa y una digital, en el caso de la versión digital lo harán en dispositivos magnéticos adecuadamente identificados.

21. Los datos que se desprendan de esta investigación no podrán ser utilizados para estudios posteriores sin la previa autorización del Ministerio del Ambiente a través de la DPNG.
22. Para el fiel cumplimiento de las obligaciones dispuestas anteriormente se responsabiliza a Diego Páez-Rosas. En consecuencia, la inobservancia de cualquiera de estas condiciones, así como el uso indebido de este documento, serán sancionados conforme la normativa aplicable y dependiendo de la infracción podría conllevar a la suspensión inmediata de la investigación.

Valoración Técnica: Steve Bayas | **Categoría:** COLABORADOR USFQ - 2022

Considerando que el Título II, numeral 2.2, literal V del Estatuto Orgánico de Gestión por Procesos de la Dirección del Parque Nacional Galápagos publicado en la Edición Especial Nº 349 del Registro Oficial publicado el martes 16 de octubre del 2012, establece entre las atribuciones y responsabilidades del Director de Gestión Ambiental "Administrar y organizar las actividades de investigación que se desarrollen en las áreas protegidas de Galápagos, en coordinación con el proceso de investigación", además que mediante Resolución Nº 71 del 14 de Diciembre del 2012, el Director del Parque Nacional Galápagos delegó al Director de Gestión Ambiental, para que en su nombre y representación tramite y suscriba los actos relacionados con el desarrollo de proyectos de investigación científica en las áreas protegidas de Galápagos; en uso de la facultad delegada y de conformidad a lo señalado en el Título Cuarto, capítulo I del Estatuto Administrativo de la Dirección del Parque Nacional Galápagos, otorga el presente PERMISO DE INVESTIGACIÓN.

Reporte de Avances o Final: 05 de febrero de 2023

Entrega de Propuesta para Renovación: 05 de febrero de 2023

FIRMA DEL INVESTIGADOR PRINCIPAL

Fecha de emisión: 24 de abril 2022

Director de Gestión Ambiental
DIRECCIÓN DEL PARQUE NACIONAL GALÁPAGOS

Sello PNG

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